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Award Number: DAMD17-02-1-0024

TITLE: Role of Androgen Receptor in Growth of Androgen  
Independent Prostate Cancer

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REPORT DATE: January 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

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# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY</b> <i>(Leave blank)</i>			<b>2. REPORT DATE</b> January 2004	<b>3. REPORT TYPE AND DATES COVERED</b> Annual Summary (15 Dec 2001 - 14 Dec 2003)
<b>4. TITLE AND SUBTITLE</b> Role of Androgen Receptor in Growth of Androgen Independent Prostate Cancer			<b>5. FUNDING NUMBERS</b> DAMD17-02-1-0024	
<b>6. AUTHOR(S)</b> Charlie D. Chen, Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> The University of California, Los Angeles Los Angeles, California 90024-1406  <i>E-Mail:</i> chenc@ucla.edu			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b> Original contains color plates: All DTIC reproductions will be in black and white.				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. ABSTRACT (Maximum 200 Words)</b> Hormone therapy is an effective treatment for advanced hormone sensitive (HS) prostate cancer. However, the treatment is short-lived and hormone refractory (HR) cancer eventually develops. Through gene profiling using seven pairs of HS and HR xenografts, we identified overexpression of androgen receptor (AR) is the only consistent change in the progression of prostate cancer. In the first grand period (01/02-10/03), we confirmed that AR protein is elevated in HR tumors. Using lentivirus and retrovirus systems, we were able to overexpress AR in HS prostate cancer cells. In vitro and in vivo experiments demonstrated that overexpression of AR is sufficient for HS-to-HR transition. We also developed a system (shRNA) to knockdown AR in HR LNCaP cells and shown that AR-knockdown abolished HR phenotype in vitro. In the last grand period (01/03-01/04), we demonstrated that overexpression of AR is required for hormone refractory prostate cancer in vivo using both LAPC4 and LNCaP cells. Although, we were unable to obtain conclusive results to determine the mechanisms for AR overexpression, we demonstrated that gene amplification is not the sole cause of the overexpression.				
<b>14. SUBJECT TERMS</b> Prostate cancer, androgen receptor, androgen independent				<b>15. NUMBER OF PAGES</b> 6
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

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## INTRODUCTION

I proposed in the grant to examine if overexpression of androgen receptor is necessary and sufficient for prostate cancer progression from androgen dependent to independence (Here the terms are changed to hormone sensitive to hormone refractory). In the first grant period, I demonstrated that overexpression of androgen receptor is sufficient for the progression in vitro and in vivo. In this grant period, I demonstrated that overexpression of androgen receptor is necessary for the progression.

## BODY

### Necessity test

#### To knockdown AR in HR LAPC4 cells by RNA interference

In the first grant period, we demonstrated that AR is required for growth of HR LNCaP cells using shRNA. To determine that the phenomenon is not specific to HR LNCaP cells, we also knocked down AR in LAPC4, another HR cell line, using the same technology. When cells were implanted into the flanks of castrated male mice, both knockdown cells (LNCaP and LAPC4) grew slower than vector-infected controls (Figure 1). Moreover, those tumors that did grow did not express GFP when compared to vector-infected controls (insets) and still expressed AR protein (data not shown), indicating selection for cells that escaped AR knockdown. These data indicate that increased AR levels are necessary for hormone refractory prostate cancer.

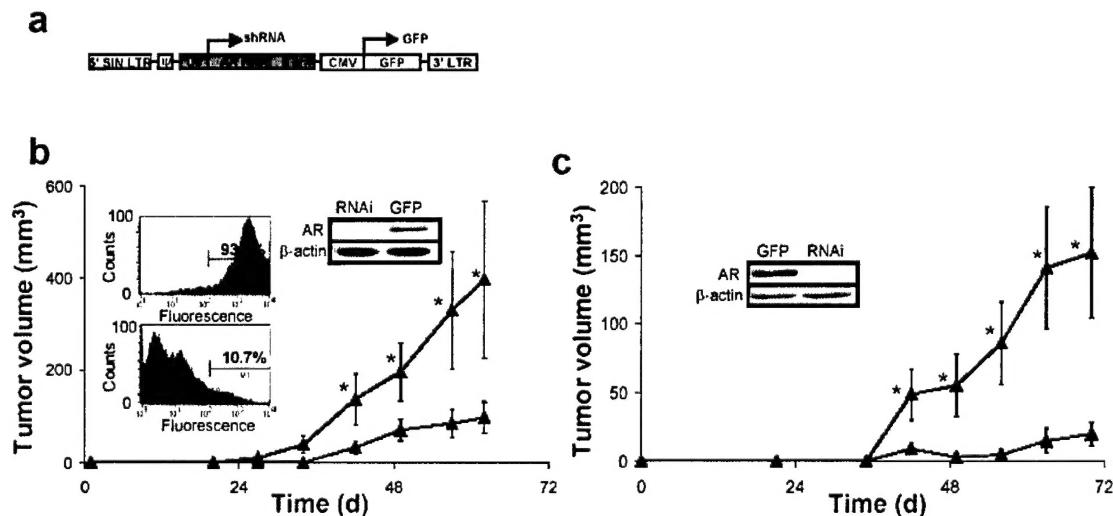


Figure 1. Androgen receptor expression is necessary for hormone sensitive-to-hormone refractory progression **(a)** Schematic of shRNA lentivirus directed against androgen receptor **(b)** Tumor volume ( $\pm$  SEM) of hormone refractory LAPC4 cells infected with either the shRNA-expressing (blue,  $n = 8$ ) or control virus (grey,  $n = 8$ ) (\* signifies  $P < 0.05$ ,  $t$ -test). Right inset, western blot of shRNA-expressing and control (empty vector) cells. Left inset, flow cytometry for GFP-positive cells of representative tumors

(control virus, top; androgen receptor shRNA virus, bottom). (c) Tumor volume (+/- SEM) in a similar experiment using hormone refractory LNCaP cells.

Mechanisms of AR overexpression. In the original proposal, we proposed to study the mechanisms responsible for the overexpression of AR in AI tumors. Up to now we are unable to generate data for the original proposed work, because we could not get enough number of single cells for each pair of xenografts. We have already tried this experiments three times. We are optimizing the digestion protocol to improve the yield.

It was demonstrated that hormone refractory prostate cancer samples have AR gene amplification (Visakorpi et al., 1995). To examine if the gene amplification can explain our xenograft finding, we initiated collaboration with the Visakorpi group in Finland. We performed the comparative genomic hybridization (CGH) on hormone sensitive and hormone refractory pairs of LAPC4, 9, and LNCaP. Using this method, we did not observe any AR gene amplification in hormone refractory xenografts compared to their respective hormone sensitive counterparts, suggesting that gene amplification cannot be the sole cause, if at all, for the overexpression.

Table 1. Comparative genomic hybridization (CGH) on hormone sensitive (HS) and hormone refractory (HR) LAPC4 and 9, and hormone sensitive LNCaP.

Sample	Gains	Losses
9HS	7p14-pter,	1p13-p21, 3p21-pter, 6cen-q22, 8p21-pter, 17q24-qter
9HR	7p14-pter,	1p13-p21, 3p21-pter, 6cen-q22, 8p21-pter, 17q24-qter
4HS	1p32-pter, 8q24, 9q34, 16p, 17q24-qter, 19, 22, Xq26-q28	1p22-31, 2q22-q34, 4p14-qter, 5p14-qter, 6cen-q24, 8q21-q23, 13q21-qter
4HR	1p32-pter, 8q24, 9q34, 16p, 17q24-qter, 19, 22, Xq26-q28	1p22-31, 2q22-q34, 4p14-qter, 5p14-qter, 6cen-q24, 8q21-q23, 13q21-qter
LNCaP HS	19p	2p13-p23, 4q26-q32, 6cen-q21, 13q21-q25
LNCaP HR	not done	not done

#### KEY RESEARCH ACCOMPLISHMENT

1. Demonstrated that AR overexpression is necessary for hormone refractory prostate cancer by RNA knockdown technology
2. Demonstrated that AR gene amplification is not the sole cause of the overexpression

## REPORTABLE OUTCOMES

None

## CONCLUSION

In the last grant period, I was able to demonstrate that AR overexpression is necessary for hormone refractory prostate cancer progression *in vivo*. We also demonstrated that gene amplification is not the sole cause of the AR overexpression.

## REFERENCES

Visakorpi, T., Hytyinen, E., Koivisto, P., Tanner, M., Keinanen, R., Palmberg, C., Palotie, A., Tammela, T., Isola, J., and Kallioniemi, O. P. (1995). *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. *Nat Genet* 9, 401-406.